

## CLAIMS

WE CLAIM:

1. A substantially purified 2-hydroxyethidium.
2. The substantially purified 2-hydroxyethidium of claim 1 wherein the 2-hydroxyethidium is at least 85% pure.
3. The substantially purified 2-hydroxyethidium of claim 1 wherein the 2-hydroxyethidium is at least 90% pure.
4. The substantially purified 2-hydroxyethidium of claim 1 wherein the 2-hydroxyethidium is at least 95% pure.
5. The substantially purified 2-hydroxyethidium of claim 1 wherein the 2-hydroxyethidium is at least 99% pure.
6. A method for detecting superoxide in a sample comprising the steps of:  
    adding hydroethidine to the sample;  
    subjecting the sample to conditions under which hydroethidine and superoxide can react to generate 2-hydroxyethidium; and  
    detecting specifically the presence of 2-hydroxyethidium in the sample wherein the presence of 2-hydroxyethidium indicates the presence of superoxide in the sample.
7. The method of claim 6, wherein the sample is a biological sample.
8. The method of claim 6, wherein the presence of 2-hydroxyethidium is detected by HPLC.
9. A method for determining the amount of superoxide in a sample comprising the steps of:  
    adding hydroethidine to the sample;  
    subjecting the sample to conditions under which hydroethidine and superoxide can react to generate 2-hydroxyethidium; and

measuring specifically the amount of 2-hydroxyethidium in the sample for determining the amount of superoxide in the sample.

10. The method of claim 9, wherein the sample is a biological sample.

11. The method of claim 9, wherein the amount of 2-hydroxyethidium is measured by HPLC.

12. The method of claim 9, wherein the amount of 2-hydroxyethidium is measured by HPLC-mass spectrometry.

13. A method for producing 2-hydroxyethidium comprising the steps of:  
dissolving Fremy's salt in a first solution containing acetonitrile and phosphate buffer to form a Fremy's salt solution;  
mixing the Fremy's salt solution with a second solution containing hydroethidine and phosphate buffer to form a reaction mixture in which 2-hydroxyethidium is generated;  
extracting 2-hydroxyethidium from the reaction mixture; and  
obtaining substantially purified 2-hydroxyethidium from the extract.

14. The method of claim 13, wherein more than 80% of hydroethidine is converted to 2-hydroxyethidium.

15. The method of claim 13, wherein more than 90% of hydroethidine is converted to 2-hydroxyethidium.

16. The method of claim 13, wherein the volume ratio of acetonitrile to phosphate buffer in the first solution is about 1 to 1, and the phosphate buffer is of about 10 mM to about 50 mM and has a pH value from about 7.4 to about 7.6.

17. The method of claim 13, wherein the concentration of hydroethidine in the second solution is from about 60 µM to about 120 µM, and the phosphate buffer of the second solution is of about 50 mM to about 100 mM and has a pH value from about 7.4 to about 7.6.

18. The method of claim 13, wherein the molar ratio of Fremy's salt to hydroethidine in the reaction mixture is about 4.5 to 1.

19. The method of claim 13, wherein the Fremy's salt solution and the second solution containing hydroethidine and phosphate buffer are mixed for about 15 minutes to about 60 minutes.

20. The method of claim 19, wherein the Fremy's salt solution and the second solution containing hydroethidine and phosphate buffer are mixed for about 25 minutes to about 35 minutes.

21. The method of claim 13, wherein an extraction solution containing chloroform and methanol is used to extract 2-hydroxyethidium from the reaction mixture.

22. The method of claim 21, wherein the volume ratio of chloroform to methanol in the extraction solution is from about 1.8 to 1.0 to about 2.2 to 1.0.

23. The method of claim 13, wherein substantially purified 2-hydroxyethidium is obtained by using a silica column to purify 2-hydroxyethidium from the extract.

24. The method of claim 13, wherein the method further comprises separating insoluble matters, if any, from the reaction mixture before the reaction mixture is extracted for 2-hydroxyethidium.

25. A method for producing substantially purified 2-hydroxyethidium comprising the steps of:

mixing superoxide and hydroethidine under conditions that superoxide and hydroethidine react to form 2-hydroxyethidium; and

purifying 2-hydroxyethidium to obtain substantially purified 2-hydroxyethidium.